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Prospecting for Adipose Progenitor Cell Biomarkers: Biopanning for Gold with In Vivo Phage Display

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In this issue of *Cell Stem Cell*, Daquinag et al. (2011) take a lesson from the oncology field to search for stromal progenitor cells within adipose tissue. Their use of phage display biopanning in vivo identified an isoform of the extracellular matrix protein decorin as a receptor for the adipokine resistin.

In the current issue of *Cell Stem Cell*, Daquinag et al. (2011) present a tour-de-force technical approach that uses phage display methods to characterize adipose tissue-specific progenitor cells in vivo. While phage display has been used extensively in the cancer field to characterize high-affinity peptides recognizing tumor cells, its application in the stromal/stem cell arena has been limited (Brown, 2010). Dr. Mikhail Kolonin, the senior author on this UT-Houston team effort, had prior expertise in the application of phage display methods to cancer biology and adipose tissue (referenced in Daquinag et al., 2011). Using high-throughput screening of a combinatorial library, he had identified high-affinity peptides recognizing surface proteins on a panel of tumor cell lines. Additionally, Kolonin and his colleagues had extended this approach in vivo, using it to identify organ selective peptides in the pancreas and to target adipose tissue for ablation. Building on this foundation, the authors here combined murine tissue-specific phage screening in vivo with flow cytometry to target novel surface markers on adipose progenitor cells (Daquinag et al., 2011). By focusing on CD34+CD31−CD45− stromal cells in adipose tissue and lung in sequential screens, they identified a peptide (WAT7) enriched in adipose tissue relative to lung by a 15-fold ratio (Daquinag et al., 2011). Confocal microscopy was used to pinpoint the co-localization of the fluorochrome-labeled WAT7 peptide to cells positive for the pericytic markers CD146 and alpha-smooth muscle actin (Daquinag et al., 2011). Using affinity chromatography and protein sequencing methods, the ligand for the WAT7 peptide was identified as the nonglyconated isoform of decorin, a secreted extracellular matrix protein associated with obesity and diabetes (Bolton et al., 2008). Taking it a step further, the authors raised antibodies against WAT7 to identify the corresponding endogenous peptide (Daquinag et al., 2011). Surprisingly, the anti-WAT7 antibodies recognized resistin, an adipokine first identified as a murine fat cell-secreted protein implicated in insulin resistance (Schwartz and Lazar, 2011). When the nonglyconated decorin protein identified as the WAT7 ligand was overexpressed in the 3T3-L1 preadipocyte cell line, it stimulated proliferation and cell migration while inhibiting adipogenesis in a resistin-responsive manner (Daquinag et al., 2011).

The development of monoclonal antibody reagents recognizing cell-specific surface antigens revolutionized the hematopoietic stem cell (HSC) field. Using selective panels of antibodies, it has been possible to map the developmental pathway for B- and T-lymphocyte lineages. Historically, the stromal/stem cell field in bone marrow and adipose tissue has continued to lag behind that of HSCs due to the lack of cell-and tissue-specific antibodies and surface markers. Multiple groups, including that of Simmons and Torok-Storb (1991), among others, developed monoclonal antibodies such as Stro-1 against bone marrow stromal cell lines, and these remain valuable and widely used reagents. Nevertheless, there remains a need for novel approaches to identify and distinguish stromal/stem cells across tissues and species. The in vivo phage display technology presented by Daquinag et al. offers a next-generation, functional approach to addressing this unmet need.

Their current manuscript focuses exclusively on the adipogenic potential of adipose tissue-derived cells (Daquinag et al., 2011). The work raises intriguing questions whether this lineage-specific cell population has direct links to multipotent mesenchymal stromal/stem cells. Mesenchymal stromal/stem cells were originally isolated in bone marrow and mesenchymal stem cells (MSCs) from this tissue source have received the greatest attention in the literature. More recently, similar populations have since been characterized in amnion, placenta, skeletal muscle, tendon, and fat, as well as other tissues. While these various cell populations share many features in common, they differ with respect to their surface immunophenotype and differentiation potential. In direct comparisons, adipose-derived adipose stromal cells (ASCs) exhibited superior adipogenesis, while bone marrow-derived bone marrow stem cells (BMSCs) showed greater osteogenesis (Sakaguchi et al., 2005). Recently, the concept of ASCs and MSCs as "perivascular" or "pericytic" cells based on their expression of CD146 and related markers has received attention (Crisan et al., 2008; Zannettino et al., 2008). Nevertheless, long-term BrdU labeling studies have localized putative progenitor cells not only to the perivascular space but also to extravascular sites adjacent to mature adipocytes within adipose tissue (Staszkiewicz et al., 2009). This background highlights the need for novel biomarkers, such as the decorin isoform described in the current paper, as potential tools to distinguish

and enrich tissue-specific progenitors which may prove to be MSC-like cells. Such reagents have the potential to accelerate the pace for the translation of MSC-like cells to the clinic, as well as to advance our understanding of their basic science at the mechanistic level. Additionally, phage display may yield peptides suitable for targeted manipulation and/or selective homing of MSC-like cells in situ. Indeed, Kolonin and colleagues have employed such approaches to selectively ablate adipose tissue in vivo (Kolonin et al., 2004).

While resistin has been studied for over a decade, its receptor and signal transduction mechanism remains an active area of investigation (Schwartz and Lazar, 2011). Few studies have reported evidence of a resistin receptor (Schwartz and Lazar, 2011). The current work provides novel evidence that resistin interacts directly with decorin. It remains to be seen if and how decorin mediates resistin signal transduction in multiple tissues. Of course, there are always caveats. For example, there are species-specific differences in the expression and functionality of resistin (Schwartz and Lazar, 2011). While resistin is associated with adipocytes in the mouse, human macrophages also express this protein, so the biology of the WAT7/decorin interaction in the mouse may not mimic that in man (Schwartz and Lazar, 2011). Still, such minor points should not detract from the bigger story, namely, that phage display methods have emerged as an unbiased in vivo discovery tool for the characterization and identification of novel surface markers and signal transduction pathways for stem cells. This method has the potential to shed new light on our understanding of how stem cells in solid organs interact with their microenvironmental niche in situ. The next challenge will be for other stem cell laboratories to reproduce and expand on the UT-Houston team's exciting approach.

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Wnt to Notch Relay Signaling Induces Definitive Hematopoiesis

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The molecular mechanisms specifying hematopoietic stem cells (HSCs) in the vertebrate embryo remain poorly understood. Recently in *Nature*, Traver and colleagues demonstrate that timed wnt to Notch relay signaling across multiple cell types serves as an early upstream mechanism of HSC induction in zebrafish (Clements et al., 2011).

Every second of our life, millions of blood cells have to be replaced to maintain our blood system. All these cells are ultimately produced from hematopoietic stem cells (HSCs), which have the capacity to give rise to all blood cell types and to self-renew in order to maintain HSC numbers life-long. Due to their fascinating ability to repopulate the entire blood system of a recipient organism upon simple transplantation, HSCs have successfully been used for regenerative cell therapy for decades. However, the low number of clinically available HSCs remains a major restriction for their application. Novel approaches for their expansion or de novo generation in vitro would therefore have a huge clinical impact.